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THE HISTOLYSIS OF THE MUSCULATURE OF CULEX PUNGENS DURING METAMORPHOSIS.[1]

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INTRODUCTION.

The phenomena attending the metamorphism of insects has attracted the attention of biologists for many years. The marked contrast which the larval form bears to the adult insect was alone sufficient, at first, to attract the observation of naturalists. Since Metschnikoff's work in 1883 setting forth the theory of phagocytosis, much has been done to determine the role of the phagocytes in the development of organisms, especially such as exhibit a transformation of form and function in their ontogenetic processes, after leaving the egg and entering upon an independent life. But in spite of much and careful study certain phases of the problem of metamorphosis are far from solved. It is the purpose of this paper to set forth the cytological phenomena associated with metamorphosis as found in *Culex pungens*, paying particular attention to phagocytosis in relation to the histolysis of the musculature. The work was done during the winters of 1902–'03 and 1903–'04 in the Zoölogical Laboratories of Syracuse University, under the directon of Dr. Chas. W. Hargitt, whose supervision of the work, as well as the valuable aid and suggestions of Dr. Smallwood, are gratefully acknowledged.

METHODS.

Material was collected during the summer months of 1902 and 1903 and fixed by several methods. Picro-sulphuric acid, corrosive sublimate and Zenker's fluid gave good results. It

[1] Contributions from the Zoölogical Laboratory, Syracuse University.

was found necessary, to insure good fixation, to puncture the chitinous integument before immersing in the respective fluids. Specimens punctured, or clipped in two or three different places with fine-pointed scissors, and then plunged at once into hot 35 per cent. alcohol gave very good results. Specimens killed and preserved in formalin gave poor fixation, and those killed in cold alcohol were also inferior to the above.

Serial sections cut three microns and stained on the slide by the iron-hæmatoxylin method were made and studied in the various stages of development. Bordeaux-red made an excellent counter-stain for these sections. To supplement these, series were stained in Delafield's hæmatoxylin and eosin.

Historical.

Although the subject of metamorphism has excited the interest of naturalists of all ages, for definite work upon the problem of the internal phenomena, one need go no further back than the work of Weismann in 1864; while it was that of Metschnikoff ('83), introducing the idea of phagocytosis, that gave the incentive for closer and more thorough investigation of the subject.

Weismann studied the postembryonic development of *Musca vomitoria* and called attention to the degeneration of the tissues of the pupa. He introduced the term "histolysis" to indicate the process of change which took place. According to him the degeneration is of a fatty nature and the resulting mass mingles with the blood whose elements have also degenerated forming altogether a thick fluid (Brei). Fragments of the mass become isolated, surround themselves with a membrane, take on a granular appearance, lose their fatty particles and acquire a nucleus. These conglomerates of detritus were termed granular spheres (Körnchenkügeln), and to them he attributes the formation of free cells *de novo*, and believes they furnish material for the construction of new tissues.

Ganin ('77) studied the muscidæ and agrees largely with Weismann. He believes that the products of degeneration serve in the formation of new organs only as nutritive substance. He demonstrated the existence and composition of the imaginal discs, which persist as embryonic rudiments in the various organs

through the growing stage, and are the centers of new growth during the process of pupation.

Viallanes ('82) describes the destruction of the fat-body, in which he finds a setting free of granules by rupture of the cell membrane. These granules constitute a sphere stained by carmine surrounded by a non-staining substance, and having a distinct outline resembling protoplasm, and are, probably, daughter-cells of the adipose cells. They resemble the embryonic cellules which constitute the outline of the first muscles of flight in the imago. The granular spheres of Weismann, Viallanes divides into two classes, the large and the small, having many or few nuclei respectively. The latter may tend to the formation of a fatty fluid (purée) on dissolution of the elements. The large ones are hypertrophied adipose cells and include the embryonic cellules.

In muscular destruction he speaks of a "regressive evolution" and "evolution by degeneration." In the former the muscle nuclei become spherical, enveloped by protoplasm and proliferate. The contractile substance disappears as if nourishing the new elements, an area of granules resulting. In the latter the nucleoli first disappear followed by a dissolution of the contractile substance.

As already stated, Metschnikoff ('83) changed the course of study in these lines by the discovery of phagocytosis. He first described certain cells of the animal body as scavengers, and gave to them the name "phagocytes." Since then much of the work done on the problem of metamorphosis has been upon this subject. Metschnikoff describes muscular phagocytes which play a part in the destruction of the muscles in the metamorphosis of insects and tad-poles. This phagocyte has for its nucleus that of a muscle fiber which becomes enlarged and surrounded by a granular protoplasm, finally breaking loose from the muscle and ingesting fragments of tissue.

Following Metschnikoff, Kowalevsky ('85) took up the work. He finds leucocytes, penetrating into the muscular substance of *Musca vomitoria* a few hours after passing into the nymphal stage, and, by their pseudopods, tearing the tissues and breaking passages for others. After incorporating fragments the leucocytes return to the circulation swollen with the ingested particles. These, Kowalevsky believes, are the same as the granular spheres

of Weismann. The nuclei of the muscles resist longer the agents of destruction and may be seen in the fragments of contractile tissue carried off, but are finally destroyed by the leucocytes and undergo fatty degeneration. At the end of two days all the leucocytes are converted into these granular spheres but continue to ingest fragments of destroyed tissue.

Originally no granular spheres are found in the young larva but the tissues are surrounded by fluid containing blood cells in large numbers. In pupæ of one or two hours there is a penetration of the blood cells into the muscle substance beginning at the head end of the body. They surround the bundles and some passing within the sarcolemma lie flat between the latter and the muscular substance, but soon send out prolongations which divide the muscles into bits. These same blood cells then ingest the fragments. After the muscles of the cephalic portion of the body have been attacked in this manner, the adipose body is the seat of a similar change. Kowalevsky has seen granules break through the surface of the salivary gland, and others coming to join them, give the appearance of a morula. At the end of forty-eight hours he saw the number of granular spheres on the surface diminish, and attributes the fact of this diminution to their penetration into the adipose cells, a process different from that found by Viallanes ('82).

Korotneff ('92) studied Tinea (*Trichonsis tonsurans*) where he finds the following to be the chief phases of metamorphosis. There are no spherical mesenchyme cells in the larva, the cœlome contains only leucocytes and granular spheres. The leucocytes take absolutely no part in the degeneration of the tissues. The formation of the imaginal muscles is considered as a reformation of the larval muscles. The fibrillar portion of the muscles becomes granular and contracted and the nuclei multiply, chiefly on one side. The granular bundles are resorbed or undergo a slow dissolution without the leucocytes taking any part in the change, while the nuclear bundle parallel to it moves off from the surface and soon produces new fibrillæ. On the other hand, he believes in the intervention of leucocytes in the histolysis of *Musca vomitoria*, for the more energetic phenomena necessitate a rapid transformation of the tissular detritus into new organs.

He compares the two modes to those which pass in chronic and acute pathological phenomena.

Bataillon ('90) found that the muscles destined to disappear in the tail of the pollywog (anoures) presented undeniable evidence of degeneration before the appearance of the phagocytes. Phagocytes seem drawn toward the place of degeneration, but not before many sarcolytes (muscular fragments, containing nuclei or not) are present, and these latter may break up without being incorporated in the phagocytes. Thus the migratory cells play only an accessory part in the process. Metschnikoff ('92) takes up the subject again and maintains in response to Bataillon, that in the muscles there is shown neither a disappearance of the nuclei, spontaneous degeneration of the muscular fasciculus, nor infiltration of the muscles by leucocytes. The atrophy then is brought about by muscular phagocytes derived from the muscular nuclei which persist and are surrounded by a granular sarcoplasm. Thus the muscular fasciculus is disassociated and absorbed by one of the elements which constitute it without any intervention on the part of the leucocytes. "Neither the disassociation of filaments nor the formation of myoplastic fragments or sarcolytes are ever spontaneously produced without the active coöperation of the muscular phagocytes." [1]

Bruyne ('98), in an admirable paper, gave a detailed account of a thorough study of the metamorphosis of *Musca vomitoria*, as well as other insects and crustaceans. He finds the muscles reduced to isolated fasciculi in which the striation is distinctly recognizable. The nuclei are hypertrophied and lodged in finely granular protoplasm. Part of the muscle bundle retains its morphological characteristics and stains with hæmatoxylin; other parts show the above phenomena. Furthermore, numerous sarcolytic fragments taking the rose tint of the eosin are found in the excavations of the muscles and about the bundle; also small ones shading in color to a pale violet. These manifestly mark a transition from the normal muscular bundle to the necrotic sarcolytes. Leucocytes do not participate in these changes. The destruction of the muscles, which is evident, has come about then

[1] "Response a la critique de M. Bataillon au sujet de l'atrophie musculaire chez les tetard," *Ann. Institut Pasteur*, 1892, p. 236.

without their intervention and before their arrival, contrary to Kowalevsky.

At the end of physiological activity of the muscles, there is a weakening and chemical alteration shown by the staining properties and minute structure, the cause of which is in the muscle itself. The chemical alteration now attracts leucocytes by chemotaxis and these ingest the sarcolytes. At a later period phagocytic leucocytes, empty or gorged, and the fragments upon which they prey, occupy the cavities of the body, and the author believes that the engorged leucocytes are identical with the granular spheres of Weismann. It is the function of these phagocytes to remove the muscular detritus from its place of origin, digest it, and transport the products of this digestion to the places where they may be utilized anew in the animal economy.

Bruyne further notes that the removal and placing in reserve of muscular debris often happens in an entirely different manner, and here he agrees with the views of Metschnikoff. In one case there was no degeneration of the nuclei followed by their incorporation in phagocytes, but on the contrary they were seen to hypertrophy before a trace of degeneration was present. The nucleus with the sarcoplasm around it isolates itself from the rest of the muscle fiber and forms a complete cell. These cells conduct themselves like the muscular phagocytes of Metschnikoff, in the larval tail of the frog, *i. e.*, acting like an amœboid cell, it ingests muscular debris. He calls them "sarcoclasts" or "myoclasts." These cells are numerous, consisting of muscular nuclei surrounded by cytoplasm which radiates in all directions to the periphery and results in a general network in the meshes of which are located the sarcolytes, the largest of which are grouped near the nucleus and retain their rose tint; the smaller, more wasted ones near the periphery show a pale staining property, which facts indicate that there is a change going on within the cell.

In *Bombyx mori* the same author describes the same phenomena as taking place. Both leucocytes and sarcoplastic phagocytes were found, the latter in a relatively small number. Similar conditions were found in other organisms studied by him.

Anglas ('00), studying the wasp and bee, found the muscles to be overrun with leucocytes as soon as the contractile property

was diminished. If present before they are in limited numbers. The phagocytic activity begins only with the physiological and chemical regression of the muscles. Morphologically there is no change, but the inertness permits us to say that it is chemically modified. The intervention of leucocytes is variable in organs of the same type, and it is evident that the cells are not indispensable.

The phenomena attending the dissolution of the fat body are not those of phagocytosis, but a digestive agent is produced which acts upon it. He terms this "lyocytosis" but leaves the mode of the process undefined, other than saying that "lyocytosis is a digestive action of a lyocyte on a cell element which, as a result, enters into cytolysis and becomes a cytolyte."[1] Later he explains in another paper, a digestion taking place by an extracellular process by means of a diastase. This is purely a chemical action brought about by a lyocytic action of neighboring tissues, but whose agents are not easy to determine.[2]

In ants, according to Perez ('00), leucocytes penetrate between the sarcolemma and myoplasm, starting about the nucleus, then between fibrils along the line of least resistance, *i. e.*, there is a leucocytic phagocytosis. Terre ('00), studying the same animals, as well as wasps, finds no such phenomena as Perez describes. He believes it impossible to make leucocytic phagocytosis play a part in the muscular degeneration. Myoblasts were described as found, and these cells and their position resemble what Perez describes as leucocytes. He gives account of two such: (1) embedded in myoplasm, (2) small ones variable in position, usually superficial, sometimes near the larger ones, and hard to determine whether or not surrounded by protoplasm. The small nuclei are found at an early period before there is the slightest evidence of the beginning of metamorphosis. In a later work of the same year he tends to agree with the idea of Anglas and states that metamorphosis may be accomplished without phagocytosis as generally understood. If the term is taken in its literal meaning of ingestion of bodies, it does not always apply, for regression of muscles and other organs may take place by an extracellular digestion. Again he says ('98) that by contact of the small

[1] "Note préliminaire sur les metamorphoses internes de la Guipe et de l'Abeille — la Lycytose," *Compt. Rendu Soc. Biol.*, LII., p. 94.

[2] "Sur la signification des termes phagocytose et lyocytose," *Ibid.*, LII., p. 219.

myoblasts the contractile substance seems to disappear as if by digestion and absorption.

Caullery and Mesnil ('00) claim a phagocytosis in the metamorphosis of crustaceans. The leucocytes arrive outside of the muscular substance at a time when there is a degeneration in the muscles as shown by the indistinctness of the striation. The myoplasm passes into a formless mass of remnants of former muscles in the meshes of a protoplasmic network in which state the nuclei resemble amœbocytes.

Needham ('00) in studying the metamorphosis of the flag-weevil (*Mononychus vuplieculu*) finds an interesting point in the fact that phagocytes do not appear in the destruction of larval tissues until the imaginal stage is entered upon. Then they appear in large numbers in the midst of the fat along the sides of the abdomen. The significance of this is more marked when one considers that the flag-weevil has a complete and rapid metamorphism.

Breed ('03) gives an account of the muscular changes in *Thymalus marginicollis* Chevr. Three groups of muscles receive separate consideration here. One strictly larval, which completely disintegrates and is lost; a second which metamorphose into imaginal muscles; and a third which are strictly imaginal, having no counterpart in the larva. These classes present different characteristics, and the muscles of one class present great individual variation. In the muscles of the second class there is a longitudinal division of the original fibers into several fibers. A destruction of the fibrillæ follows until the muscle fiber becomes a structureless mass of sarcoplasm. The number of nuclei is greatly increased by amitotic division. At an early stage cells derived from the intracellular tracheoles are found between the fibers, which eventually give rise to the new tracheoles. In the muscles of the first class he makes two divisions, one of which undergoes a progressive atrophy with no change of the nuclei until the last stages when they undergo a typical chromatolysis; in the other there is a process similar to that described above for muscles of the second class, *i. e.*, the muscles undergo a change similar to those of the metamorphosing muscles until the stage of reconstruction begins. *Tracheal* cells are found in both of these cases, more especially in the latter, which the author believes

disappear into the blood stream and become imaginal leucocytes. He says " the degeneration of the larval muscles is entirely chemical, there being no evidence of phagocytosis." [1]

MUSCULAR DEGENERATION IN CULEX.

In the study of the phenomena attending the postembryonic development, or metamorphosis, of *Culex*, one should first consider certain facts in the life history of the insect. Metamorphosis is complete — the insect belonging to the holometabola. Its larval life has as average duration of nine to ten days, and it passes the pupal stage in three days. This, however, is not constant and one cannot judge the stage of development of a larva or pupa by the number of days elapsed since emergence from the egg. In isolating a group of eggs in a dish of water one finds pupæ at the surface as early as the seventh or eighth day, perhaps, while not all will have reached that stage of their existence until several days later. One then can only judge from the size of the larva as to its stage of development.

The outward manifestations of metamorphism throw little light upon the internal phenomena attending such change. Several moults may mark the steps of development through the larval and pupal life of an insect and the periods of time between such ecdyses show no change in the external morphology though the internal changes are constantly going on. This fact is well accentuated in *Culex*, for while the pupa is looked upon as being the stage of transition from the larva to the imago, the histolysis of the tissues does not correspond to this period. Notwithstanding the fact that metamorphism is complete and rapid, *Culex*, unlike the holometabolic insects so far studied, does not go into a resting stage at the end of larval growth. The pupa is very active. What then shall be expected? Certainly the larval muscles cannot all degenerate and be destroyed during the pupal stage if the insect still is moving its body through the function of these organs. Is there first a new growth of muscles for the imago before the degeneration of the larval fibers? If such were the case we should look for the phenomena of degeneration in the imago and not in the pupa.

[1] "The Changes which Occur in the Muscles of the Beetle During Metamorphosis," *Bull. of Mus. of Comp. Zoöl. Harvard*, Vol. XL., No. 7, p. 373.

These questions are all answerable to a greater or less degree after a study of sections of wrigglers from the time they are two thirds grown until they are ready to emerge. Degeneration begins as early as the two thirds stage and is found first in certain muscles of the thorax. The next are those of the head, and lastly in the abdomen extending distally from the thorax in order. The appearance of imaginal organs is also made in the larva, the thoracic appendages and their muscles beginning to form before the advent of the pupal stage. Thus in one series of sections one may study the degeneration of larval muscles and the regeneration of the imaginal ones. Destruction is not complete in the abdominal segments even in late pupal life, and it is possible, indeed probable that this is completed in the adult insect.

As to the initial cause of the degeneration of the larval muscles, it has been shown that authors disagree. Kowalevsky has made the attacking leucocytes or blood phagocytes (hæmatophages) directly responsible for the disintegration, claiming that they surround the muscle bundle, insert pseudopods into the sarcolemma and so break their way into the substance. The fibers are broken up in this manner and the fragments ingested by the hæmatophages. Bruyne, on the other hand, while admitting phagocytic leucocytosis as an important factor in the disintegration of muscles destined to disappear, does not find them the initial cause of the phenomena. He affirms that muscles lose their striations, become fragmented and that the nuclei hypertrophy before there is an appearance of leucocytes. He believes the cause is to be looked for in the muscles themselves, which degenerate at the end of physiological activity.

It seems indeed probable that, metamorphosis having been established in a group of insects, the phenomena of degeneration, or at least of atrophy, of the organs of the adaptive life, which are not to serve the adult, should occur when the limits of that stage of the life are reached and such organs become inactive. In such a case these organs may be looked upon as foreign bodies and the phagocytes performing their accustomed office, set about their removal. This seems the more probable since the degenerating tissue would attract leucocytes by chemotaxis. This must be modified somewhat in the case of *Culex*, in which

there is no resting stage. Does the cessation of physiological activity mark the beginning of degeneration here? It cannot be otherwise; the muscles which first disappear are those of the head and thorax which would be of as little use to the pupa as though there were a true resting stage; the other muscles are not lost until late. Any muscle must certainly have ended its physiological function before it is removed by what can be considered a normal process.

Further generalizations will be deferred until the conditions observed in *Culex* have been described. The muscles of the larva, before degeneration has begun, show the usual characteristics of muscular tissue in other insects. Bundles of coarsely striated fibers, each with its sarcolemma and nuclei, are arranged longitudinally along the segments of the body. The nuclei are oval and lie close to the contractile substance within the sarcolemma. Immediately surrounding it is a small amount of finely granular protoplasm. With the onset of regressive change in these muscles, there is an increase in the amount of the granular material about the nucleus and the latter becomes separated from the contractile substance, loses its oval shape and hypertrophies. This takes place before there is any appreciable change in the contractile substance. Later the striations are less distinct and become gradually lost, while at the same time the fibers become divided longitudinally into fibrillæ. The sheath of the bundle is stripped away from the fibers by an increasing amount of granular material, in which the hypertrophied nuclei come to lie. All this takes place without the appearance from other sources, or formation from preëxisting elements in the muscles, of active cells of any kind.

From this stage of regression several phases of degeneration present themselves, none of which are constant, nor confined to any one set of muscles. The nuclei lying free in a mass of granular material continue to hypertrophy. The chromatin threads are early lost, and small deep-staining granules appear in the nuclear substance which later become arranged about the periphery close to the membrane. The nucleolus disappears at this time, and the center of the nucleus may be almost transparent. Finally the membrane ruptures and the granules become scattered

in the general tissue detritus. In some cases there is evidently a limited multiplication of the nuclei by direct division at the beginning of degeneration but the products of such a division pursue a course of disintegration not unlike that just described. In fact no direct evidence of such a division can be here given except the relatively large numbers of nuclei found in some instances at an early stage of regression, and all of these come to the same termination.

Along with this degeneration and disappearance of the nuclei there are changes in the contractile substance. The ensheathing layer of the bundle becomes separated and often broken by the increase of the granular material in which the nuclei lie. The fibers become reduced to fibrillæ and fragmentation and dissolution follow. Often in the same material with the nuclei between the outer sheath and the fibers are seen small round cells. These may make their appearance before the striation of the muscles is lost, but never before there is an abundance of the granular material outside of the bundle and never before the nuclei are hypertrophied. They have never been seen to attack the fibers. They have, undoubtedly, a phagocytic function as will be shown, but play a secondary part in the process, for degeneration is well marked before their appearance.

The place of origin of these small round cells is indicated by such conditions as are shown in Plate X., Fig. 4. In the connective tissue surrounding and lying near the muscle are found cells of an elongate form, often overlooked in the fine threads. Though a division of these cells has not been actually observed, a transition from the elongate cell lying between the fibers of connective tissue, to the round cell in the granular material about the muscle seems quite probable. Such cells engage themselves with the ingestion and removal of the products of degeneration, about the muscle bundle, and later they are seen filling the body cavity, in whole or in part, much swollen with particles showing various stages of degeneration.

In other instances such phagocytes are not found at all. There is a regression of the muscles to fibrillæ and a limited amount of the granular material is found. The muscular substance then seems to disappear as if dissolved away by some chemical action.

Here the sheath about the bundle, not being stretched away or broken by the increase of material within, remains longer than the contractile substance showing the original limits of the muscle. The nuclei in such cases pass through the same chromatolysis as in the above instances.

The late stages of muscular degeneration are seen in the body cavity. Fragments, either carried there by phagocytes, or swept there by some other agent, undergo a fatty degeneration which will be described later. Having thus briefly considered the phenomena found in *Culex*, a discussion of the various points and a comparison with the results obtained by others may be made.

It is the primary factor in this muscular degeneration which appears difficult of determination and upon which authors have expressed materially different opinions based upon observations upon the same or different animals. Kowalevsky ('85) notes the active *interference* of phagoctes within five to six hours after the change to the nymphal stage (*Musca vomitoria*), commencing in the first segments of the body where there is no indication of degeneracy in the muscles; nucleus, sarcoplasm, and striations are absolutely normal from a morphological standpoint. The progress is rapid so that by the seventh or eighth hour all the muscles of the first segment are destroyed.

Bruyne ('98) in the study of the same animal, agrees with Kowalevsky as to the time of the appearance of leucocytes. In nymphs of one and two days, entire muscles were reduced to a conglomerate mass of angular fragments of variable dimensions but having preserved their striations. Almost all are enclosed in leucocytes, while many of the latter, still empty, are found between the fragments of muscular debris. He defines these leucocytes as little protoblasts moving in an amæboid manner, pushing out their pseudopodial extremities through the sarcolemma and into the muscular substance. However, Bruyne does not consider this the beginning of the degeneration, but describes areas showing at once a transition from the muscle fasciculi, still striated, with nuclei in different stages of hypertrophy, to fragmentation of the contractile substance and change in the staining properties of the sarcoplasm, all of which occur without

the presence of leucocytes. He brings this forward to show that the beginning of degeneration is marked by morphological and chemical changes and not by leucocytes. This view which is also upheld by other observers denies that the initial cause of muscular degeneration is phagocytosis, but that phagocytosis is secondary to some other change in the muscle itself.

In *Culex* the appearance of phagocytes about a muscle is a comparatively late occurrence. In the majority of cases there is a considerable loss of substance in the contractile tissue, a vacuolation, without their presence and often they are not found at all. What Bruyne notes in *Musca* and represents in Fig. 1, Pl. VII., of his work, is repeatedly found in this insect, *i. e.*, the muscles show an early degeneration by separation of the fibers and hypertrophy of the nuclei as already shown. The first change noted is a stripping off of the ensheathing layer which often becomes broken, and an increase in the amount of the granular protoplasm which surrounds the nucleus, separates it from the fibers, which, together with a more granular appearance of the sarcoplasm, presents a condition comparable to what is known to pathologists as "cloudy swelling" or "granular degeneration." The nucleus leaves its normal position close to the fiber, where it presents a flattened or oval shape, to lie free in this granular material, and, at the same time, it becomes more spherical in shape. The next step is the loss of striations which gradually takes place, and a division of the fiber into fibrillæ before the form of the fiber is lost. In some instances there is a transverse breaking of the muscle bundle and a localized condensation of the contractile substance as shown in Fig. 2, Pl. X. This is probably due to a contraction of the weakened muscle and is not a constant feature of the process. In Fig. 1, Pl. X., no phagocytes are found. Several nuclei, apparently normal, except for their shape and position, are seen separated from the bundle, and n^1 shows a beginning hypertrophy.

It is evident that in *Culex* there is a direct degeneration of the muscles. Although there is in some cases, not in every instance, a phagocytosis, as will be shown later, it shows no active relation to the degenerative process either in point of time of its occurrence or in the manner in which it manifests itself. Korotneff

('92), Bataillon ('90), Needham ('00) and Breed ('03) all find that phagocytes play no part in the degeneration proper of muscles. Phagocytosis may, or may not occur, but whenever it does it is always a secondary process. No one of these authors attempts to explain the primary cause of the degeneration, though it is variously described as a morphological, physiological and chemical change. Such a change may be accounted for in the subcatobolic conditions involved in a deficient nutritive supply. At the end of physiological activity of the larval organs of an holometabolic insect the nutritive supply is diverted from these organs to the parts which are to evolve the new organs of the imago. Such a process would result in the phenomena above described, a condition which is amply illustrated in certain pathological changes in the tissues of higher organisms when the nutrition is interfered with.

The further phenomena attending the dissolution of the muscles is not constant. As above stated, there appears, in the maiority of cases, a dissolution of the muscle substance accompanied by a hypertrophy of the nuclei. In such cases the striation is early lost, the nuclei take an oval or spherical appearance and stain less regularly. In fact the most marked evidence of early degeneration among the larval muscles of *Culex* is the hypertrophy of the nuclei and changes in their morphology and staining properties. Instead of showing a chromatin network and an evenly stained nuclear substance, there are larger or smaller deep-staining granules which soon become arranged about the periphery and contained within a nuclear membrane. The nucleolus disappears and the whole nucleus breaks up by rupture of the membrane and the granules become scattered. Plate X., Figs. 2, 3 and 4, show the evolution of this process. The complete destruction by rupture and dissociation of the granules has been repeatedly seen. Such are the small dark staining bodies found scattered among the other detritus in the body cavity late in the process of degeneration. Their fate is then similar to that of other fragments of muscle tissue to be described later.

In the present study no other condition of the nuclei was observed than that of total disintegration by rupture of the membrane after the entire nuclear substance had become condensed into a few irregular granules at the periphery.

Along with this change in the nuclei there is a rapid destruction of the contractile-substance as above described. In one case by a gradual dissolution, and in the other by the advent of phagocytic cells. In the former instance there is a loss of substance without visible cause, and similar to the phenomena of lyocytosis by which Anglas ('00) accounts for the destruction of larval muscles. It would seem that some unseen agent was producing a digestive action upon the muscles in question. This is illustrated by the conditions represented in Plate X., Fig. 2. This process is marked all through the period of muscular degeneration in all parts of the body, and is undoubtedly the chief factor in the destruction of these organs. What cells are active in secreting an enzyme for the digestion of these inert organs, and why it manifests a selective action upon such tissues is difficult of explanation.

It has been shown that the degeneration of the muscles may be seen making rapid progress without the aid or intervention of phagocytes as contended by authors already cited. These wandering cells, however, make their appearance, and, at times, in considerable numbers, but in proportion to the amount of degeneration taking place their number is indeed small and inadequate to the work to be performed. In fact, it may be said that phagocytes play no part in the degeneration of the muscles in a true sense. After the muscle has become altered in its morphological, physiological, and chemical characteristics, as shown by its loss of striation and separation of nuclei, by its inertness, and by its changed reaction to dyes, and even broken to fragments either by inherent properties of the tissues themselves or by some chemical agent within the body, the debris becomes a foreign body. Wandering cells of the body which have a phagocytic action, whether blood cells or specialized cells derived from mesoblastic tissue, will set about the removal of such foreign material, and in so doing will only be performing their usual physiological function.

The origin of phagocytic cells in *Culex* is not to be attributed to the blood or to the free formation of cells in the degenerating tissues, but to the multiplication of small cells of the connective tissue which are not of a highly specialized character. Plate X.,

Fig. 4, and Plate XI., Fig. 1, besides showing the conditions above described, present several small cells in the granular material about the muscle lying free with the nuclei. They are probably the first appearance of phagocytes, and in this instance, it will be noted, they have entered before the fibers have lost their striations. If one examines the former figure the origin of these cells becomes plainly evident. In the sheath surrounding the bundle are several small elongated cells. Some of these appear large and more spherical, and show by their position and morphological characteristics, their relation to the spherical ones within the sheath though cell division has not been observed. It seems very probable that the phagocytes of *Culex* are derived, not from the blood or myoclasts of Bruyne, but from special mesodermic cells which arise by a proliferation of the cells existing in the body tissues. These cells are later found much larger because of the detritus which they have incorporated, and pass into the body cavity where we shall examine them more carefully a little later.

Of the authors who do not attribute to phagocytosis the initial cause of muscular degeneration, Korotneff ('92) and Bataillon ('90) describe phagocytes as playing a secondary part in the destruction of the debris as described in *Culex*. Breed ('03) says that there may be such a condition but does not note it in his work, while Needham ('00) claims the total absence of phagocytes until the imaginal stage. Breed says the degeneration of the larval muscles is entirely chemical, there being no evidence of phagocytosis. The tracheal cells which he describes, and their ultimate function is quite comparable to the phagocytes above described in *Culex*. In the one the cells are derived from preëxisting cells in the tracheoles and in the other from preëxisting cells in the connective tissue. Both the tracheal cells and the connective tissue cells are of mesodermic origin not specialized to particular functions, and just such cells as one would expect would give rise to phagocytic cells. Again one may find analogous cases in pathology. In the study of inflammatory changes in various tissues a proliferation and phagocytosis is very often observed. The cells which become phagocytic are derived in most instances from either connective-tissue cells or endothelial cells.

Only rarely do the proliferated epithelial cells become phagocytic and then they are of a low, undifferentiated type; they are the flat cells which resemble the endothelial cells and probably perform much the same function. The epithelial cells lining the alveoli of the lungs and of Bowman's capsule of the glomeruli of the kidney may proliferate in certain inflammatory processes and the resulting cells take up a phagocytic function.[1] This would seem to suggest the probable origin of phagocytes and their relation to the muscular destruction in *Culex*. They arise from a proliferation of mesodermic cells, appear after the degeneration has become established and have no causative relation to the degeneration.

Another point which should be mentioned, although not found in *Culex*, is that of the myoclasts described by some observers. Metschnikoff ('83) describes muscular phagocytes in the resorption of the tail of tadpoles, as noted in a preceding paragraph. In *Musca*, Bruyne ('98) also finds instances of the same phenomenon and terms these cells myoclasts. In these instances there are the same initial phenomena as described in the beginning degeneration. The nuclei, however, do not disappear after hypertrophy, but persist and become surrounded by sarcoplasm. This then becomes an amœboid cell and acts as a phagocyte. These myoclasts become very large when distended with numerous sarcolytes. In such a case we have cells arising from the degenerating tissue which attacks tissues of similar origin. The muscles having come to an end of their usefulness, produce the agents of their own destruction. In the study of *Culex* the behavior of the nuclei as above described was constant, *i. e.*, both nuclei and sarcolytes are eventually consumed together in the body cavity. Nothing that corresponds to the description of myoclasts has been observed.

Late in the process of metamorphosis, seen especially in advanced pupæ, but to some extent in earlier forms are masses of sarcolytes or muscle fragments, some free, others enclosed in phagocytes, which show various degrees of degeneration. A very few retain their normal staining properties when treated.

[1] "Proliferation and Phagocytosis," F. B. Mallory. From *The Jour. Exper. Med.*, Vol. V., No. 1, 1900, p. 7.

with hæmatoxylin and eosin. From the blue, they pass through a violet to a rose color which markes the necrosis of tissue. Some then give a copper-colored appearance, and finally clear spaces indicate the completion of fatty degeneration. The inference here is that the muscle fragments ingested by the wandering cells are carried into the body cavity where they line the body wall, and complete their work. All through the body beneath the hypodermis, and especially between the muscles of the thorax, large numbers of these cells aggregate, where they are seen scattered among free sarcolytes. Figs. 3 and 4 of Plate X. show the condition just described, Fig. 4 being farther advanced. In some instances a nucleus is found centrally located, while at other times it is pushed to the periphery and flattened to the membrane of the cell by the enclosed fragments. Degeneration seems to take place more rapidly about the nucleus and works gradually to the more distant inclosures.

Two forms of degeneration have been noted, both of a digestive nature, the one extracellular by unseen agents, as if by means of fluids secreted by cells at a distance, or in the muscle itself or some part of it; the second an intracellular digestion which occurs in the instance of ingestion of muscular debris by phagocytes. In either case the result is the same, and probably in both the products are further used in the animal economy in the rebuilding of imaginal tissues. Sarcoplasm and nucleus are both involved in this digestion, though the latter seems more resistant to this agent of destruction and dark staining granules of nuclear material are found late scattered through the body. In connection with this it should be stated that other investigators agree that not all sarcolytes or muscular debris are incorporated in phagoçytes. Bruyne ('98) says that many sarcolytes break up without being acted upon by phagocytes. He quotes Loose as giving statistical estimation that there are from 90 per cent. to 96 per cent. free sarcolytes; 4 per cent. to 6 per cent. surrounded by a plasmic envelope, and that 3 per cent. may be found in a plasmic area bearing a nucleus.

In brief, in the process by which the larval muscles degenerate and are destroyed, it has been found that the degeneration proper is a chemical one unaided by any physical action by cells outside

the muscle itself, though it is possible that the muscle is influenced by internal secretions of other cells in the body which reach them through a circulating medium. There is no leucocytic phagocytosis. Phagocytes which apparently arise from the mesodermic tissues, appear in some instances and remove the debris of muscles already broken down, and complete in part, the work already begun by other agents. There is nothing to indicate a myoclastic phagocytosis, or that the muscle gives rise to any organized elements which destroy the remaining tissue by an autophagocytosis, or which go to build up new tissues. The end of the muscle substance is that of conversion into fat or other nutritive material by a process of digestion or chemical change.

Muscular Regeneration.

The manner in which the imaginal muscles arise to take the place of those destroyed has not received as much consideration from investigators as the subject of degeneration. However, as regards those who have studied that part of the process of metamorphosis which has to deal with the development of organs of the imago, a diversity of opinions exists nearly equal to that found in the writings of those interested in the degenerative process. Bruyne ('98) notes the regeneration in *Bombyx mori.* He quotes Viallanes, and reports the same phenomena, *viz.*, a multiplication of muscular nuclei with considerable rapidity, in such a way as to determine areas of little nuclei, sometimes irregularly grouped, sometimes arranged in linear series. He notes mitotic figures here and there, but believes that both direct and indirect division intervene together. A small amount of the sarcoplasm of the larval muscles persists with these nuclei and increases. The subsequent separation of the young nuclei and the striation of the protoplasm contributes to the renovation of the muscular tissue. These cells he terms "myoblasts." Thus in the larval muscles he finds, not only the agents of destruction which he describes as "myoclasts," but also the starting point of the new muscles in the "myoblasts"; so that these two classes of cells, both products of the larval tissues, play a role, physiologically speaking analogous to the osteoclasts and osteoblasts of the higher animals.

Breed ('03) accounts for the wing and leg muscles of the imago by a direct metamorphosis. Carrying the degeneration to a few cylindrical strands of undifferentiated sarcoplasm which contain many nuclei undergoing rapid amitotic division, there is a period of little change followed by the appearance of the fibrillæ of the adult muscle. A little later the elongated nuclei which have formed by the direct division disappear and short oval nuclei are found scattered through the muscle substance. As for the histogenesis of imaginal muscles which are not present in the larva, he gives no definite information. They are probably derived from cells which resemble tracheal cells but have a different origin.

The conditions found in *Culex*, as regards these points, seem rather to confirm the views set forth by Korschelt and Heider ('99). The destruction of the larval muscles is complete. New muscles form from embryonic rudiments present in the imaginal discs. A thickening of the cells at points along the under surface of the hypodermis is found in the thorax, from which, after the degenerative process is well advanced, cells proliferate and extend into the thoracic cavity. These cells lie in irregular masses, but soon form linear series as they extend inward. Mitotic figures have been frequently noted among them. The protoplasm of these cells is not distinct, but large oval nuclei arranged in the line of their long axis appear soon to lie in a syncytium which assumes the shape of a muscle fiber. This condition is represented in Figs. 5 and 6 of Plate XI. Already a longitudinal striation is somewhat apparent in the former, but this becomes more marked in a later stage as shown by the latter figure. Here the amount of protoplasm is increased very considerably and the nuclei are pressed to the side of the fiber, and instead of being the most prominent part of the structure, often show little more than a granular material forming a line along the side of the fiber. The greater number of these nuclei atrophy and disappear at a later period, while the protoplasm continues to increase. An advanced stage which occurs late in pupal life and marks the completion of the morphological characteristics of these muscles by the appearance of transverse striæ is represented in Fig. 4, Plate XI. The nuclei have all disappeared except those which

are to be the permanent nuclei of the adult muscle fiber. It would appear in some rare instances as though several nuclei of an earlier stage coalesced to form the mature nucleus, but it was not observed in a sufficient number of cases to state that such is the case.

This process of development of the new muscles from imaginal discs is not only well shown by the sections studied, but seems the logical source of new tissues when one considers the general process recognized in embryology of organic development among insects of complete metamorphism.

General Conclusions.

1. The degeneration of the muscles begins in the thorax when the larva is two-thirds grown, and is due to chemical alterations. Phagocytosis is not a determining factor, though a lyocytosis may be present.

2. Phagocytes appear in the muscular detritus after the muscle is far degenerated, attracted by a chemotaxis, and remove a part of the necrotic tissue.

3. The phagocytes are not blood cells, but probably of mesodermic origin, formed by a proliferation of cells scattered through the connective tissue.

4. The completion of muscular destruction is the digestion of the sarcolytes either in the phagocytes (intracellular) or by a fatty degeneration of the fragments which are not ingested by cells, or by a digestion by means of a fluid secreted by cells elsewhere in the body (extracellular).

5. There is no autophagocytosis of muscles. No myoclasts are present, while, on the other hand, the nuclei undergo a disintegration and destruction along with the contractile substance and in a similar way.

6. The regeneration of new muscles of the adult is from the imaginal discs by a proliferation of the embryonic cells which have persisted undifferentiated during the larval growth.

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DESCRIPTION OF PLATES.

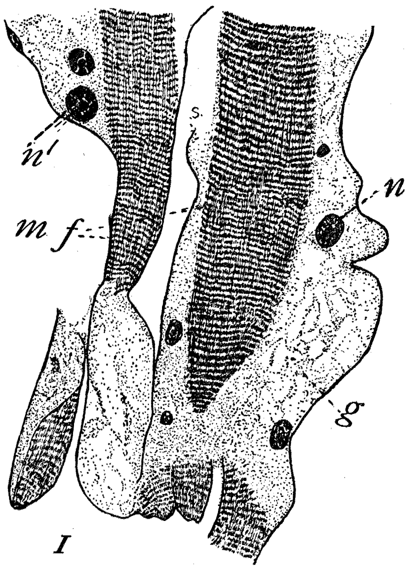
EXPLANATION OF PLATE X.

FIG. 1. *Culex pungens*, larva two thirds grown, showing the beginning of degeneration. Several nuclei (*n*) are seen in the granular material (*g*) separated from the muscle fibers (*mf*). *n′* shows a hypertrophy and nearly spherical form. The smaller nuclear bodies are shown in preceding and following sections to be similar nuclei to those shown in this section.

FIG. 2. The muscle reduced to fribrillæ with marked breaking and condensation of parts. Many hypertrophied nuclei are also seen.

FIG. 3. Here is represented a marked degree of disintegration of the muscle substance and vaculation without the presence of granular material.

FIG. 4. This shows the same as Fig. 1 with many small cells in the connective tissue envelope of the bundle.



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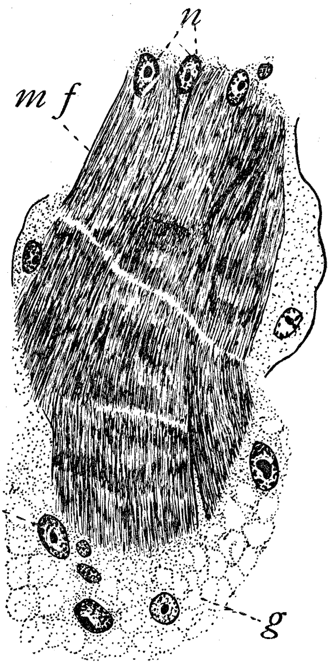

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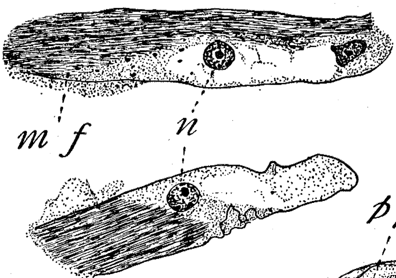

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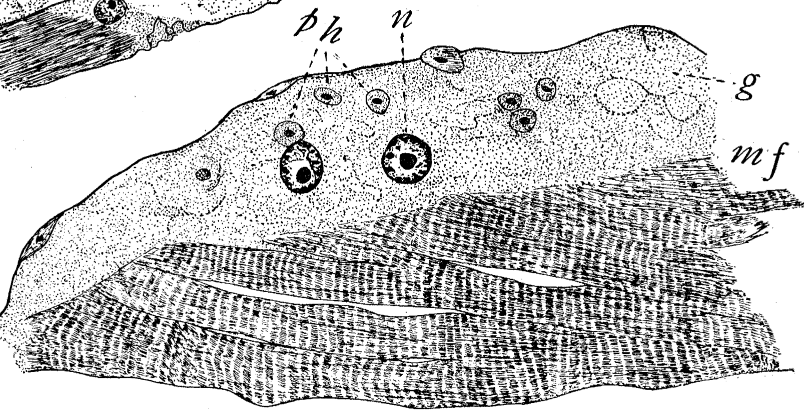

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Explanation of Plate XI.

Fig. 1. Representing a small portion of a muscle surrounded by an increased amount of granular material in which are seen, besides a single enlarged and degenerate nucleus *n*, several small mesodermic phagocytes *ph*.

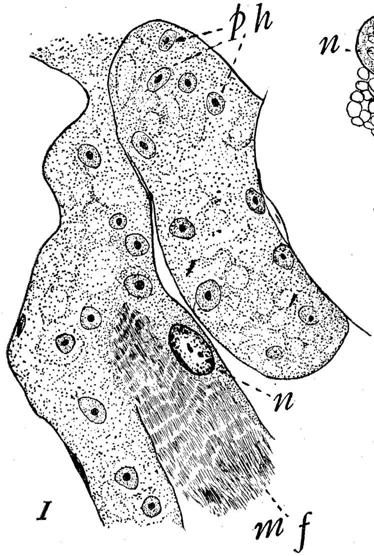
Fig. 2. The section shows a mass of sarcolyted and engorged phagocytes, the former undergoing destructive changes both within and without the cell.

Fig. 3. Similar to Fig. 2, though showing several isolated phagocytes with phagocytes *ph* with nuclei either centrally placed or crowded to the periphery. To the left is seen a formless mass of detritus.

Fig. 4. Showing final stage of the regenerated muscle fiber with striations.

Fig. 5. Showing the beginning of the formation of the new muscles of the adult. A proliferation of cells from the imaginal disc and the arrangement of the protoplasm into the form of fibers.

Fig. 6. This shows a later stage than Fig. 5, with an increase in the amount of protoplasm and the arrangement of the nuclei to the sides.



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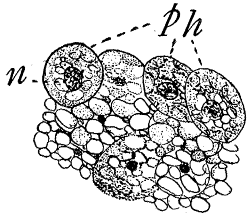

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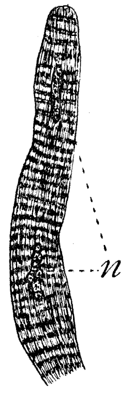

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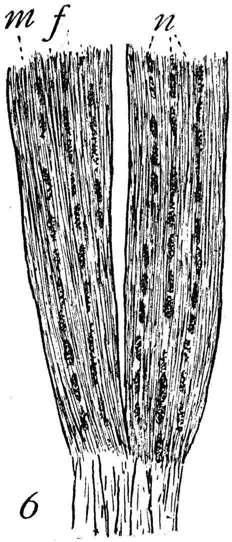

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